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Three-dimensional electrochemical immunosensor for sensitive detection of carcinoembryonic antigen based on monolithic and macroporous graphene foam

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ABSTRACT

A high performance three-dimensional (3D) electrochemical immunosensor was developed for sensitive detection of the tumor biomarker, carcinoembryonic antigen (CEA). Monolithic and macroporous graphene foam grown by chemical vapor deposition (CVD) served as the scaffold of the free-standing 3D electrode. Immuno-recognition interface was fabricated via simple and non-covalent immobilization of antibody using lectin-mediated strategy. Briefly, the well-known lectin macromolecule (*concanavalin A*, Con A) monolayer was functionalized on 3D graphene (3D-G) using in-situ polymerized polydopamine as the linker. Then the widely used horseradish peroxidase (HRP)-labeled antibody (anti-CEA) in immunoassays was efficiently immobilized to demonstrate the recognition interface via the biospecific affinity of lectin with sugarprotein. The 3D immunosensor is able to detect CEA with a wide linear range (0.1–750.0 ng ml^{-1}), low detection limit (~90 pg ml^{-1} at a signal-to-noise ratio of 3), and short incubation time (30 min). Furthermore, this biosensor was used for the detection of the CEA level in real serum samples.

1. Introduction

Early clinical diagnosis of cancer is the key to a higher survival rate. Tumor markers are macromolecules existing in tumor cells or host body fluids, which are produced by tumors associated with cancers. It has been proven that sensitive detection of tumor markers is of great significance in early detection and monitoring recurrence of cancers (Kitano, 2002; Srinivas et al., 2001). Till now, numerous immunoassays for tumor marker have been developed (e.g., enzyme-linked immuno-sorbent assay (ELISA) (Voller et al., 1978; Yates et al., 1999), chemiluminescence (Fu et al., 2006, 2008), and mass spectrometric immunoassays (Niederkofler et al., 2001; Hu et al., 2007)). Among those different techniques, electrochemical immunosensors have received a particular attention because of their simple instrumentation, fast response time, and portability (Tang et al., 2011; Guo et al., 2011; Luo et al., 2014; Kanso et al., 2014). Current electrochemical immunosensors, however, are often limited by complex detection scheme, poor sensitivity and stability, and long detection time. For instance, many immunosensors rely on two or three protocols for signal amplification to improve sensitivity (Du et al., 2011; Begard et al., 2014). The antibodies immobilized via covalent reaction, nanomaterial adsorption or polymer entrapment may compromise the detection efficiency due to low activity or poor long-term stability. In addition, the incubation time might be long due to low diffusion of macromolecules.

Over the past few years, it has been proven that electrode material and architecture play critical roles to achieve a simple, sensitive and stable detection in protein-based bioanalysis (Zhang et al., 2011; Ojeda et al., 2014). The applications of three dimensional (3D) macroporous electrodes are highly desirable since they can improve the performance of electrochemical sensors (Wang et al., 2014; Si et al., 2013; Yang et al., 2013; Dong et al., 2012a). For instance, the large internal surface and macroporous morphology improve the density, accessibility as well as the stability of the

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bioactive affinity ligands (e.g. antigen or antibody). In addition, interconnected open porosity of the host 3D electrode facilitates the kinetic diffusion and mass transfer of macromolecules (Xi et al., 2013). Among those significant progress made in building 3D electrode, the fabrication of macroporous Au electrodes was dominated. Current 3D macroporous Au electrodes, however, were mainly prepared by precisely controlled electrochemistry process (Patel et al., 2013). As the morphology of the resulting 3D Au film was difficultly controllable, the electrode was easily irreproducible. Due to its fascinating electronic and chemical properties, graphene exhibited immense potential for the development of novel and ultra-sensitive electrochemical biosensors (Liu et al., 2012; Wang et al., 2012; Feng et al., 2011). Recently, 3D monolith of graphene foam with unique macroporous structure has been grown via CVD (Chen et al., 2011). Compared with the conventional 2D (planar) electrochemical electrodes, such 3D graphene (3D-G) electrodes are attractive for the construction of ultrasensitive chemical sensors due to the unique advantages of macroporous scaffold, large active surface area, unhindered substance diffusion, high conductivity and stability (Xi et al., 2013; Liu et al., 2012; Dong et al., 2012b, Huang et al., 2010). More importantly, 3D-G electrodes provide potential for cheap and large-scale preparation with high reproducibility. As so far, few biosensors are developed based on 3D-G foam grown by CVD. This is mainly because the 3D-G foam is free-defectable and exhibits ultrahigh hydrophobicity, which imposes difficulties in its biological functionalization and application. It is well known that amphipathic polydopamine (pDA) formed by in-situ polymerization of dopamine in alkaline condition is able to adhere well onto a wide range of hydrophobic or hydrophilic surfaces (Lee et al., 2007; Morris et al., 2009). Additionally, pDA provides abundant reactive handles that can conjugate with a wide spectrum of thiol- or amine-containing molecules (e.g., protein) via Michael addition or Schiff base reactions (Cheng et al., 2013; Morris et al., 2009). Therefore, the biofunctionalization of 3D-G via pDA as the universal linker might open up the possibilities to develop new platform for three-dimensional electrochemical biosensing.

In the present work, a novel three-dimensional electrochemical immunosensor with high performance is developed using 3D-G foam as a monolithic and macroporous carbon electrode. Sensitive detection of the tumor biomarker, carcinoembryonic antigen (CEA), is achieved via immuno-recognition interface immobilized on lectin monolayer. Briefly, 3D-G electrode was firstly functionalized with a well-known lectin, *concanavalin A* (Con A) using in-situ polymerized polydopamine as the linker. Then, the immunosensing interface is efficiently fabricated by immobilizing HRP-labeled anti-CEA antibody (HRP-Ab) via biospecific lectin–sugarprotein interaction. The detection lies in the signal changes of the solution-phase redox probe in differential pulse voltammetry (DPV) after the capture of CEA to the anti-CEA interface. Owing to the 3D architecture, extraordinary properties of graphene and indirect antibody immobilization, the immunosensor demonstrates outstanding performance in terms of detection range, sensitivity, response time, and stability even no complex strategy for signal amplication is used.

2. Experimental

2.1. Reagents and apparatus

Dopamine hydrochloride (98%), bovine serum albumin (BSA) and *concanavalin A* (Con A) from *canavalia ensiformis* (Jack Bean) were purchased from Sigma-Aldrich (USA). Horseradish peroxidase (HRP) (E.C.1.11.1.7, 250 U mg^{-1}) was purchased from Shanghai Sanjie Biotechnology Co., Ltd (Shanghai, China). Carcinoembryonic antigen (CEA), HRP labeled anti-CEA antibody (HRP-Ab) and prostate specific antigen (PSA) were purchased from Keyuezhongkai Biotech Co., Ltd. (Beijing, China) and were stored at 4 °C before use. β-D-(+)-Glucose was purchased from Beijing Chemical Reagent (Beijing, China). All other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared by using ultrapure water (18.2 MΩ cm^{-1}) from a Milli-Q Plus system (Millipore).

Scanning electron microscopy (SEM) images were obtained at 5.0 kV on a JSM-6700F (JOEL, Japanese) field emission scanning electron microscope. Static contact angles were measured on a drop shape analysis system G10/DSA10 contact angle system. Electrochemical measurements were performed on a CHI 660D electrochemical analyzer (Shanghai CH Instrument Company, China).

2.2. Preparation of 3D-G based immunosensor and the biosensing process

As previously described (Xi et al., 2013), 3D graphene foam was synthesized by CVD with nickel foam being the growth substrate. After growth, the nickel substrate was removed by incubation with 3 M HCl at 80 °C for 12 h. The freestanding 3D-G foam (0.5 cm × 0.5 cm, 1 mm thick) was then fixed onto a glass slide. The electrical lead was made by silver paint and copper wire insulated with silicone rubber. Similar to the work previously reported (Lee et al., 2007; Morris et al., 2009), polydopamine (pDA) was then coated onto a 3D-G electrode by immersing this electrode into a dopamine monomers solution (1 mg ml^{-1} in 0.05 M PBS solution, pH 8.5) for 30 min. After rinsing away the unbound pDA, the 3D-G/pDA electrode was immersed into a *concanavalin A* (Con A) solution (0.5 mg ml^{-1} in 10 mM PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$, pH 7.5) for 40 min, followed by removing non-specifically adsorbed Con A via thorough rinse. Afterwards, the 3D-G/pDA/Con A electrode was dipped in a HRP-labeled anti-CEA antibody solution (HRP-Ab, 100 μg ml^{-1}) for 1 h. By this step, anti-CEA antibody was captured on Con A to demonstrate CEA recognition interface through the biospecific interaction between Con A and HRP. Then the modified electrode was dipped into an HRP solution (1.0 mg ml^{-1} in 10 mM PBS, pH 7.5) for 1 h to block the free sites of both Con A and the modified materials. After rinsing with distilled water, the obtained immunosensor (3D-G/pDA/Con A/HRP-Ab) was stocked in PBS (0.05 M, pH 7.0) at 4 °C before use.

For the biosensing of CEA, the immunosensors were incubated with various concentrations of CEA antigen (Ag) at 25 °C. After forming antigen–antibody complex, the detection was performed by measuring the inhibited current of $Fe(CN)_6^{3-/4-}$ using differential pulse voltammetry (DPV). The ratio of I/I_0 was evaluated, where I_0 represented the blank peak current, and I represented the current after each detection. To investigate the selectivity of the fabricated immunosensor, interference study was performed using bovine serum albumin (BSA), prostate specific antigen (PSA), horseradish peroxidase (HRP) and glucose. A 3.0 ng ml^{-1} of CEA solution containing 30.0 ng ml^{-1} of interfering substances was measured by the immunosensor using the above sensing protocol.

2.3. Electrochemical measurements

Electrochemical measurements were performed on a CHI 660D electrochemical analyzer (Shanghai CH Instruments, China). A conventional three-electrode system was used consisting of a bare or modified 3D-G working electrode, an Ag/AgCl (saturated with KCl) reference electrode, and a platinum disk auxiliary electrode. The solution of $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1, 1.0 mM containing 0.1 M KCl) was used as the supporting electrolyte for all electrochemical